

## CAMP test Detected *Staphylococcus delphini* ATCC 49172 β-Haemolysin Production

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### Abstract

Through a CAMP test, we first observed a *Staphylococcus delphini* strain (ATCC 49172) to release β-haemolysin. Production of the latter in this coagulase-positive species of the '*Staphylococcus intermedius* Group', in fact, has been labeled to be undetermined, thus far. Of course, a wider number of strains have to be investigated in order to define whether this property is constitutive (like in *Staphylococcus (pseud)intermedius*), or strain-dependent (like in *Staphylococcus aureus*), and which clinical impact it has; nevertheless, we can state that *S. delphini* ATCC 49172 indeed produces this toxin.

**Key words:** *Staphylococcus delphini*, β-haemolysin, CAMP test

In 1944, Christie and Atkins observed that arrow-shaped haemolysis (called "candle flame-shaped" or "arrowhead") occurred when *Streptococcus agalactiae* (group B streptococcus, GBS) was grown in a zone of *Staphylococcus aureus* β-haemolysin activity (Christie *et al.*, 1944; Darling 1975). This phenomenon was later called 'CAMP test', after the names of authors who first studied it (Christie, Atkins, and Munch-Petersen) and is currently used in microbiology laboratories as one among the most reliable methods to identify GBS, *Listeria monocytogenes* and *Rhodococcus equi* (Darling 1975; Munch-Petersen *et al.*, 1945; Ramsey *et al.*, 2010; Savini *et al.*, 2011).

To perform the assay, reference *S. aureus* strains that produce β-haemolysin are streaked perpendicularly to the tested isolate, and arrowheads are observed after 24 h incubation. Indeed, any β-haemolysin-producing *S. aureus* isolate may be used (Darling 1975); among coagulase-positive microorganisms other than *S. aureus*, nevertheless, β-haemolysin is constitutively produced by *Staphylococcus (pseud)intermedius* (Ramsey *et al.*, 2010; Devriese *et al.*, 2005), while production by *Staphylococcus delphini* (that forms, together with *S. (pseud)intermedius*, the '*Staphylococcus inter-*

*medius* Group') is labeled as undetermined (Devriese *et al.*, 2005; Savini *et al.*, 2013; Van Hoovels *et al.*, 2006; Varaldo *et al.*, 1988).

Therefore, we carried out a CAMP test by streaking *S. delphini* strain ATCC 49172 on a sheep blood plate perpendicularly to *S. agalactiae* (identification confirmed through latex agglutination) (Ramsey *et al.*, 2010) and observed arrowhead (Fig. 1), surprisingly, meaning *S. delphini* β-haemolysin production. As a confirmation, strain ATCC 49172 was cultivated on horse and rabbit blood media, where the dark, *S. (pseud)intermedius*-like, α-haemolytic band (which is β-haemolysin-related) was not formed; it was instead clearly visible on sheep blood (Darling 1975; Dinges *et al.*, 2000; Savini *et al.*, 2013).

β-haemolysin, in fact, has been known to be highly haemolytic for sheep but not rabbit and horse erythrocytes, and is neither lethal in mice nor dermonecrotic in guinea pigs (Darling 1975; Dinges *et al.*, 2000). It is secreted into the culture medium as an exotoxin by certain *S. aureus* strains, particularly those from animal habitats, as well as from all *S. (pseud)intermedius* isolates (Dinges *et al.*, 2000; Savini *et al.*, 2013). Although its role in disease pathogenesis is not completely

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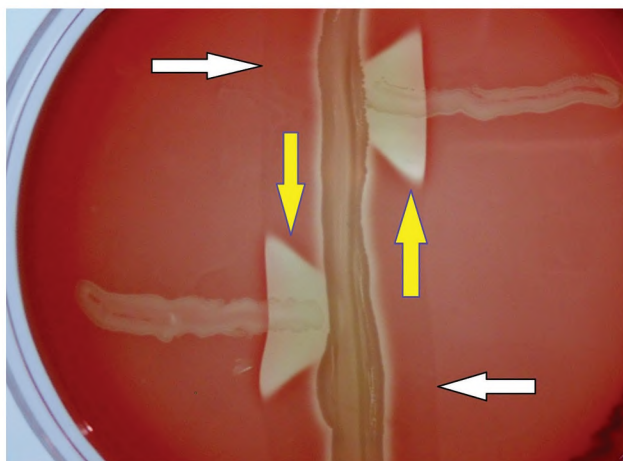


Fig. 1 - CAMP test with *S. delphini* (ATCC 49172).

Horizontal inocula: GBS strains; vertical inoculum: *S. delphini* A (ATCC 49172) – yellow arrows indicate zones of CAMP reaction; white arrows indicate edges of the  $\beta$ -haemolysin-related incompletely haemolytic band ( $\alpha$ -haemolysis).

understood, thus far, high level expression in veterinary strains seem to indicate that producing organisms garner selective advantages from this toxin secretion (Dinges *et al.*, 2000).

We first showed that an *S. delphini* strain produces  $\beta$ -haemolysin; however, this species is rarely isolated and further studies on a wider number of strains are needed, as soon as they are collected and identified, to define whether production is constitutive (like in *S. (pseud)-intermedius*) or strain-dependent (like in *S. aureus*). Nevertheless, we suggest, for the moment, that *S. delphini*  $\beta$ -haemolysin production be no more considered to be undetermined, but potential, and observed.

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#### Conflict of interests statement

The authors have no conflict of interest to declare.

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